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Reactive intermediates and carbohydrate fragmentation in Maillard chemistry

H. Weenen

Quest International, Huizerstraatweg 28, 1411 GP Naarden, The Netherlands

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Much research is devoted to the elucidation of mechanisms in the Maillard reaction. Model studies with reactive intermediates and ^{13}C-labelled precursors have contributed significantly to our understanding of flavour formation in the Maillard reaction. Several examples are discussed here: The formation of methyl pyrazines and 2-acetyl-1-pyrroline, the role of ARP's and deoxyglycosones and the formation of carbohydrate fragments from reducing sugars, 3-deoxyglucosone and ARP's. It is concluded that carbohydrate fragments, and also flavour substances derived therof, are formed from the starting reducing sugars (or the corresponding imines), deoxyglycosones, and possibly ARP's. A general scheme for flavour formation in the Maillard reaction is proposed. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Flavour ingredients based on Maillard chemistry are generally referred to as process flavourings. These are mainly used to obtain the flavour characteristics of thermally treated foodstuffs, such as meat, coffee, chocolate, nuts, potato chips and crackers.

The main challenges in Maillard chemistry for the flavour industry are to improve the quality of process flavourings, and the efficiency of preparing them. The quality of a process flavouring is determined mainly by its flavour, but also by its taste, stability, colour and its performance in the end-product. The nutritional aspects should also be considered, and the formation of toxic substances such as quinoxalines from creat(in)ine should be controlled (Jägerstad *et al.*, 1983). The efficiency of preparing a process flavouring can be improved by increasing the volume efficiency, the molar efficiency, and the atomic efficiency or atom utilization.

To further improve the quality and manufacturing efficiency of process flavourings, a better understanding of the underlying chemical reactions is required, in particular those which lead to high flavour impact ingredients. To this end charm analysis (Acree *et al.*, 1984) or aroma extract dilution analysis (Grosch, 1993, 1994) of thermally processed foods, or process flavourings (Hofmann, 1995) is very useful. To elucidate the mechanisms underlying the formation of substances of interest, model systems, in particular with the use of labelled precursors are quite useful (Weenen *et al.*, 1994; Tressl *et al.*, 1994). The role of reactive intermediates is also particularly important in the Maillard reaction. Amadori rearrangement products (ARPs) and Heyns rearrangement products (HRPs) (Yaylayan and Huyghues-Despointes, 1994) but also deoxyglycosones (Weenen *et al.*, 1992; Weenen and Tjan, 1994) are relatively stable intermediates, and their role is still under debate.

The formation of these reactive intermediates may also be of importance for the stability of the end-product. Little is known about the stability of process flavourings, other than that spray drying is normally used to improve their stability. New encapsulation techniques may contribute to the stability of process flavourings (Karmas and Karel, 1994).

Another aspect which deserves more attention, is the interaction of Maillard reaction precursors with other components present in the reaction matrix. In particular the effect of lipids on the formation of volatiles is being studied extensively (Farmer *et al.*, 1989), and shows that lipid oxidation products, in particular aldehydes, can be incorporated into volatile end-products.

An alternative approach to improve manufacturing efficiency, is to use experimental design techniques to optimize process flavourings, and the use of new manufacturing technologies, such as extrusion (Izzo *et al.*, 1994), and the use of microwave heating (Peterson *et al.*, 1994).

FLAVOUR IMPACT COMPONENTS OF PROCESS FLAVOURINGS

The flavour impact components of a series of thermally processed foods have been determined by Grosch

(1994). While previous literature on the volatiles of processed foods indicates that there are hundreds to more than a thousand identified volatiles in a single processed food, such as coffee or meat (Nijssen *et al.*, 1996), the results of aroma extract dilution analysis indicate only a few dozen flavour impact components (Blank *et al.*, 1992; Gasser and Grosch, 1988).

The first example of a systematic study of the flavour impact components of a process flavouring is reported by Roberts and Acree (1994). The character impact components of the aroma produced by the reaction between glucose and proline at 200°C were determined using Charm analysis. The four most important flavour impact components were found to be diacetyl, 2-acetyltetrahydropyridine, 2-acetyl-1-pyrroline and 2,5-dimethyl-4-hydroxy-3(2H)-furanone. The flavour impact components of cysteine-containing process flavourings have been determined by Hofmann and Schieberle (1995). The flavour impact components identified by them, are very similar to those identified by Grosch (1994) in beef, however a few compounds were discovered which have not (yet) been detected in processed foods, i.e. 2-(1-mercaptoethyl)furan, 5-acetyl-2,3-dihydro-1,4-thiazine, 5-propionyl-2,3-dihydro-1,4-thiazine, 3-hydroxy-6-methyl-2(2H)-pyranone and 2-(1-mercaptoethyl)thiophene.

MODEL STUDIES

One of the first studies in which ^{13}C-labelled precursors were used to contribute additional mechanistic information on the formation of flavour substances in the Maillard reaction, was on the formation of methylpyrazines (Weenen *et al.*, 1992, 1994). NMR was used to determine the content and position of the the ^{13}C-label in methylpyrazines formed when glucose and fructose were allowed to react with asparagine at pH 8.0. When 1-^{13}C-glucose was used as precursor, the ^{13}C-label was detected exclusively in the methyl (CH_3) or methine (CH) carbon atoms, in about equal amounts. No label was detected in the quarternary carbon atom of the methylpyrazines. This is in agreement with the mechanisms shown in Scheme 1 and Scheme 2.

Recently, the formation of 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine has been the subject of a number of studies. A mechanism for the formation of 2-acetyltetrahydropyridine was first proposed by Hodge *et al.* (1972). They proposed that Strecker degradation of proline and pyruvaldehyde yields 2-acetyltetrahydropyridine. Schieberle (1990) reported that citrulline was also a good precursor for 2-acetyltetrahydropyridine. In addition Schieberle found that ornithine, proline and citrulline are all effective precursors for 2-acetyl-1-pyrroline.

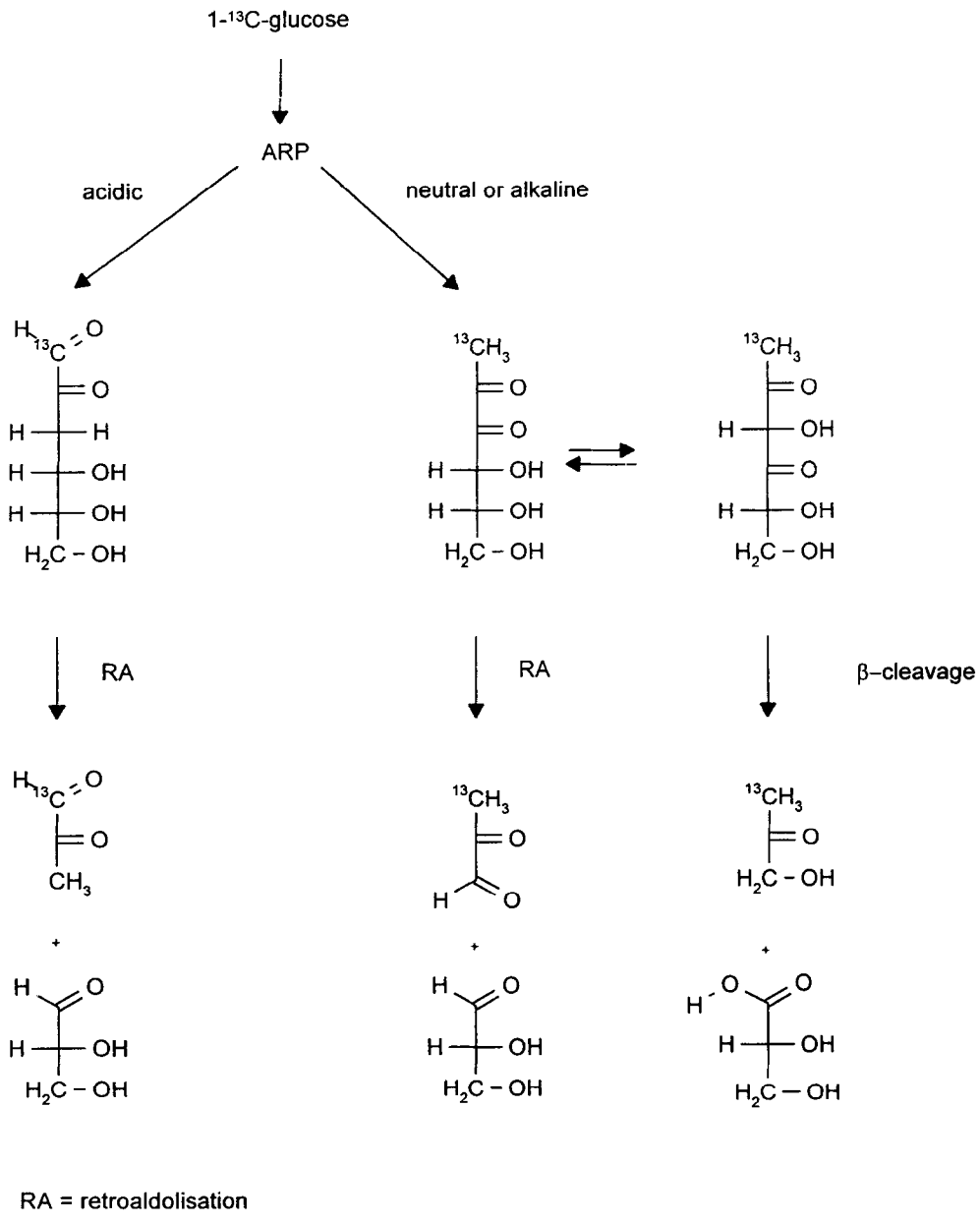
Of particular interest are the studies of Rewicki *et al.* (1993), in which they used glucose with a ^{13}C-label at the C-1 position and proline as precursors (Scheme 3). When they analysed the position of the ^{13}C-label in 2-acetyl-1-pyrroline that was formed in this reaction, it was found that only 50% of the product contained the ^{13}C-label, and this was exclusively in the 2′-position. The explanation given for this phenomenon was that the intermediate 1-deoxyglucosone isomerizes to 3,5-dihydroxy-2,4-hexanedione which is symmetrical. Starting from 1-^{13}C-glucose, the label is then at the C-1 position, but fragmentation can take place from either side.

REACTIVE INTERMEDIATES: AMADORI REARRANGEMENT PRODUCTS

Whereas Amadori rearrangement products are well studied, little has been published on Heyns rearrangement products (Yaylayan and Huyghues-Despointes, 1994). Amadori rearrangement products have been reported to occur in various thermally treated foodstuffs, but not much has been published on the decomposition of Amadori rearrangement products into volatiles (*vide infra*: carbohydrate fragmentation). Another question which has not really been answered, is whether Amadori rearrangment products are essential intermediates in the Maillard reaction, or whether they are relatively stable side products, which do not contribute much to the flavour impact components of process flavourings (Molero-Vilchez and Wedzicha, 1997).

REACTIVE INTERMEDIATES: DEOXYGLYCOSONES

Deoxyglycosones have long been postulated as important intermediates in the Maillard reaction. Anet (1964) has done some interesting work on the chemistry of 3-deoxyglucosone. He synthesized 3-deoxyglucosone from difructoseglycine, and studied its conversion to 5-(hydroxymethyl)-2-furfural and metasaccharinic acid. Kato (Kato, 1960 and Kato *et al.*, 1989) isolated several 3-deoxyglycosones as their bis-2,4-dinitrophenylhydrazones under Maillard reaction conditions, and established their role in melanoid formation and protein cross-linking. Edwards and Wedzicha (1992) sudied the kinetics of the reaction of 3-deoxyglucosone with sulphite, mercaptoethanol and glutathione. In all three reactions, the rate-determining step is the spontaneous conversion of 3-deoxyglucosone to a reactive intermediate (3,4-dideoxyhexosulos-3-ene), followed by a rapid reaction of this intermediate with the nucleophile. Much less has been published on the chemistry of 1-deoxyglucosone, undoubtedly because it is apparently very difficult to synthesize (Ishizu *et al.*, 1967). The formation of 1-, 3- and 4-deoxyglucosones from Amadori rearrangement products has been demonstrated elegantly by Beck *et al.* (1989), who trapped these glycosones using 1,2-diaminobenzene. The authors proposed



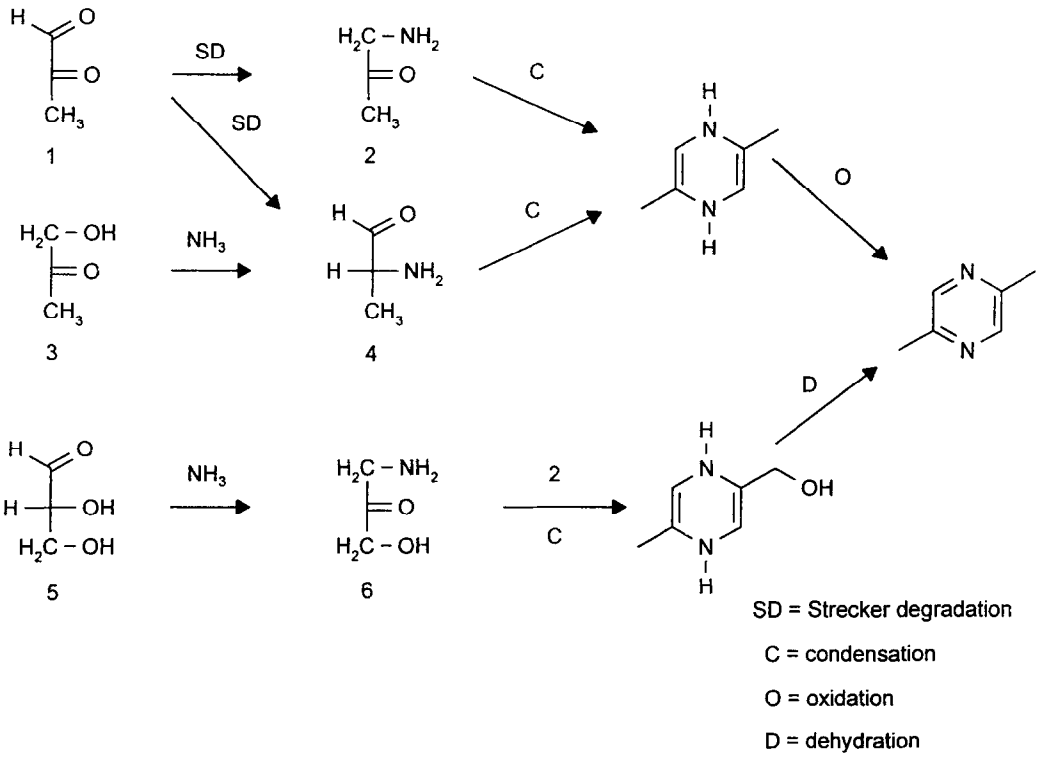
Scheme 1. Formation of pyruvaldehyde, glyceraldhyde and hydroxyacetone from 1-^{13}C-glucose.

two furanoside and two pyranoside structures for 3-deoxyglucosone, which were later proven to be incorrect, or at least insignificant by ourselves (Weenen and Tjan, 1992, 1994).

3-Deoxyglucosone was prepared by us using a method developed by Khadem *et al.* (1971), which was later optimized by Madson and Feather (1981). To purify the 3-deoxyglucosone prepared this way, an hplc method was developed by Weenen and Tjan (1992) using a silica gel bonded β-cyclodextrin phase. Using acetonitrile/water as the mobile phase, a single broad peak at 200 nm UV detection was obtained for 3-deoxyglucosone. At 250 nm however, the peak representing 3-deoxyglucosone was a clear double peak, indicating multiple isomers. The purified 3-deoxyglucosone showed no siginificant carbonyl absorptions in the IR spectrum, and its mass spectrum indicated a molecular ion of 162, which is in agreement with the molecular formula $C_6H_{11}O_5$. It was concluded that 3-deoxyglucosone should therefore consist of isomeric (hemi)-acetal/ketal bicyclic structures (Scheme 4). NMR studies were in agreement with these proposed structures, and suggest that isomers such as 4 or 5 (Scheme 4) are predominant in aqueous 3-deoxyglucosone.

CARBOHYDRATE FRAGMENTATION

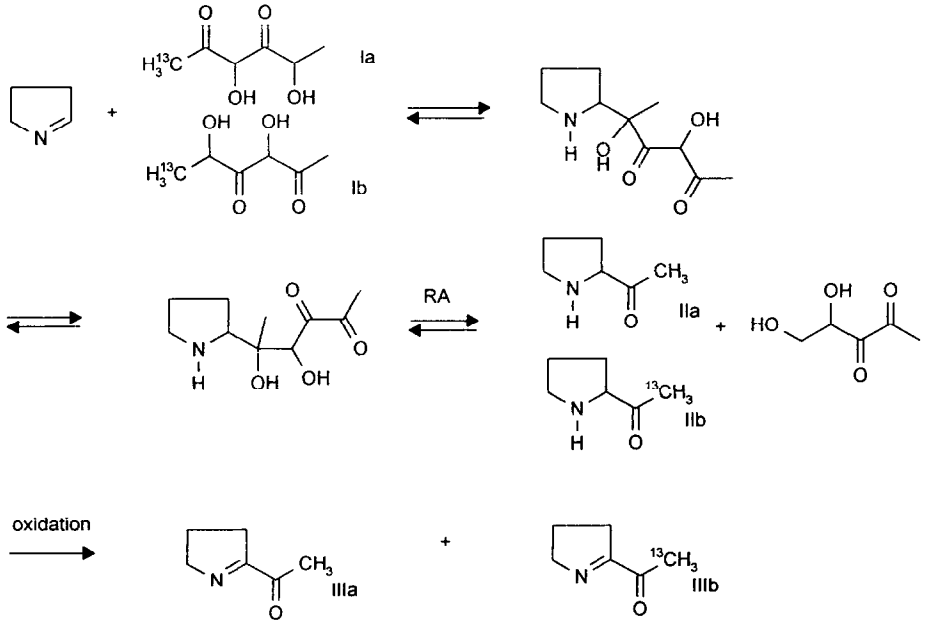
Flavour substances generated in the Maillard reaction can be divided into either of two classes from a chemical point of view: they are formed by condensation of the intermediate deoxyglycosones, or they are formed after



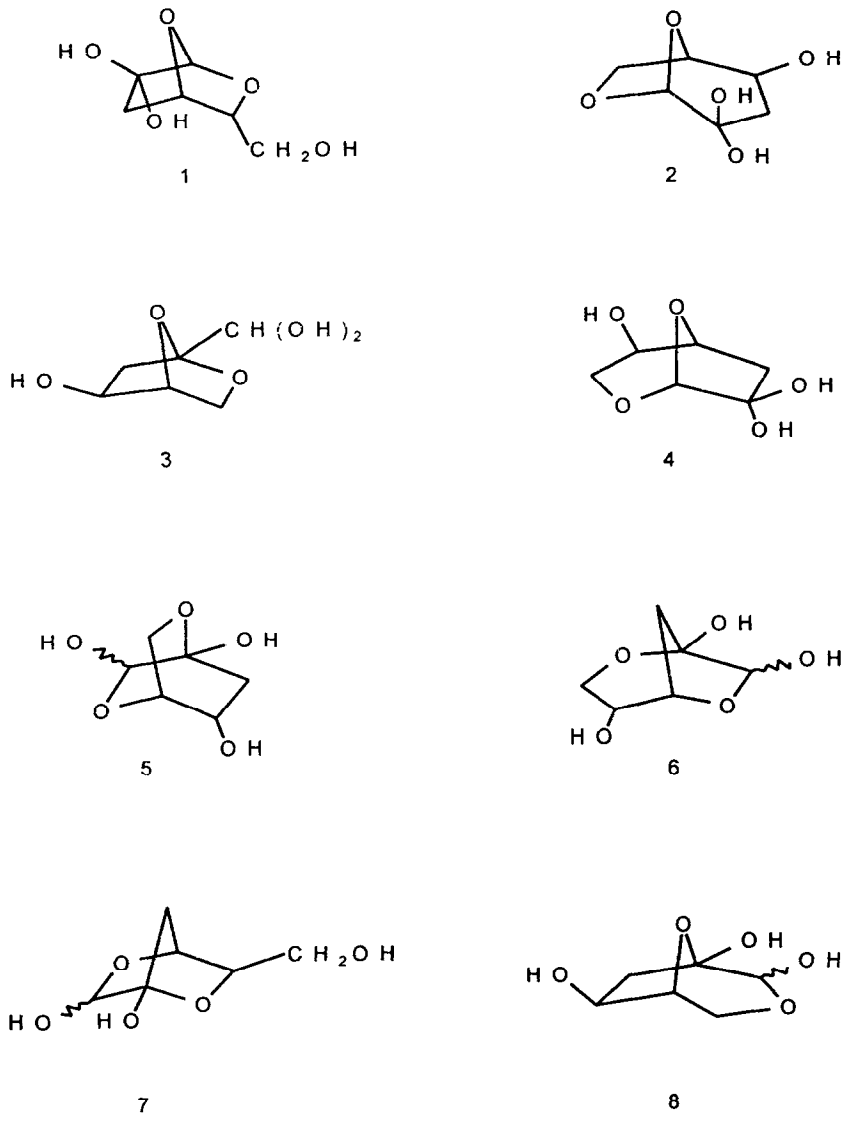
Scheme 2. Formation of 2,5-dimethylpyrazine from C_3-fragments.

carbohydrate fragmentation. Products formed by cyclization/condensation of the intermediate deoxyglycosones include 2,5-dimethyl-4-hydroxy-3(2H)-furanone, 2-methyl-4-hydroxy-3(2H)-furanone, furfural and 5-(hydroxymethyl)-2-furfural, and are often formed in high to very high yields. Products formed after carbohydrate fragmentation, include pyrazines, thiazoles, carbocyclic compounds and other heterocycles, and are always formed in low to very low yields. Since carbohydrate fragmentation seems to be the yield-determining step in the formation of these compounds, carbohydrate fragmentation was studied in more detail by us.

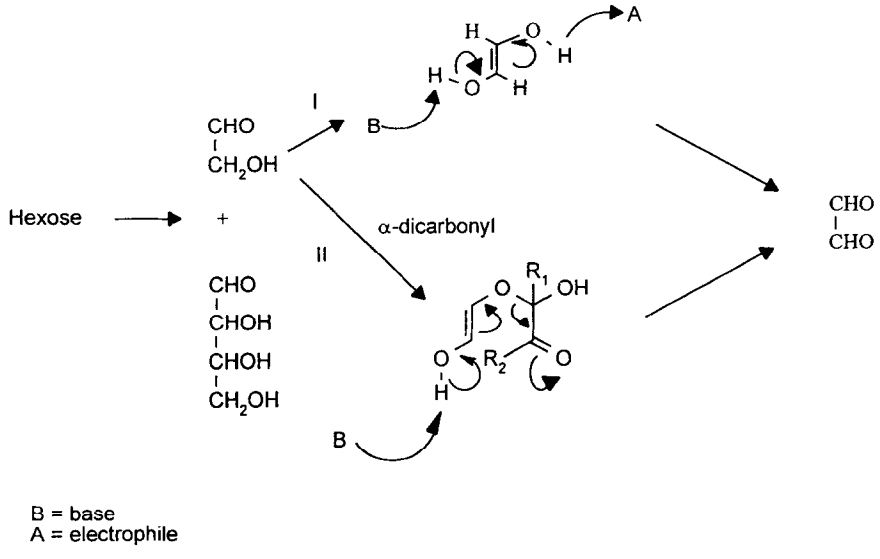
First of all cysteamine was used as a trapping agent for carbonyl-containing intermediates (Weenen and Tjan, 1994). The most striking result of this study was that hydroxyacetone was formed from glucose and



Scheme 3. Formation of 2-acetyl-1-pyrroline from glucose and proline according to Rewicki *et al.* (1993).



Scheme 4. Bicyclic structures of 3-deoxyglucosone.

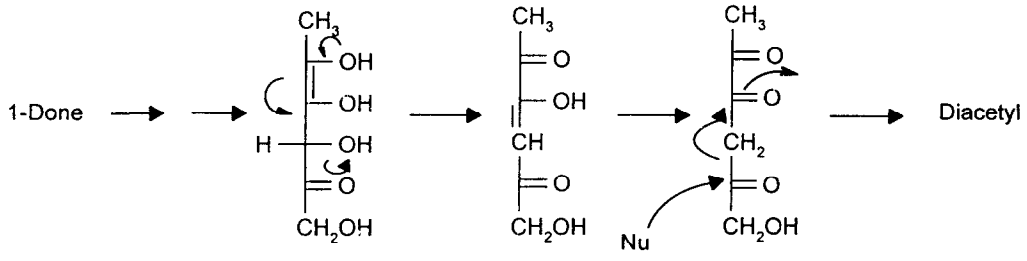


Scheme 5. Formation of glyoxal.

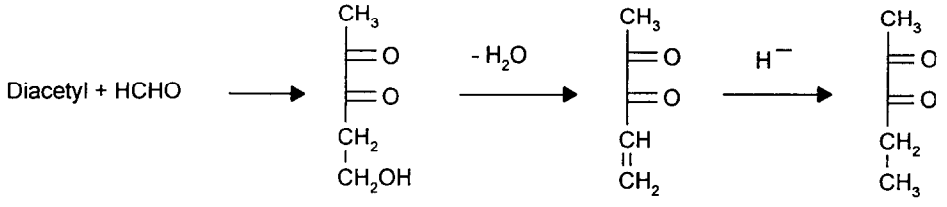
fructose, but not from 3-deoxyglucosone. We explained this by postulating that 1-deoxyglucosone, once formed from glucose and fructose, isomerizes to 2,4-dioxa-3,5,6-trihydroxyhexane. This latter compound is a β-dicarbonyl substance which can easily undergo β-cleavage to give hydroxyacetone, as indicated in Scheme 1.

Secondly, 1,2-diaminobenzene was used as a trapping agent, which selectively traps α-dicarbonyl-containing substances. Various carbohydrates (0.55 M) and the Amadori rearrangement product of glucose and alanine (0.55 M) were reacted with cyclohexyl amine (0.6 M), alanine (0.6 M) and without amine/amino acid, in an aqueous phosphate buffer solution (30 ml, 0.33 M, pH 8) and *n*-butanol (15 ml) containing 4-methylquinoline as internal standard, at 100°C for 1 h (Weenen and Apeldoorn, 1996). Four α-dicarbonyl substances were observed as volatile quinoxaline derivatives: glyoxal, pyruvaldehyde, diacetyl and 2,3-pentanedione. The formation of pyruvaldehyde can be explained as resulting from retro-aldolization of 1- and 3-deoxyglycosones, as shown in Scheme 1. Glyoxal cannot be explained as originating from deoxyglycosones, nor from Amadori rearrangement products; however, it seems to be formed directly from aldoses or the corresponding imines by retro-aldolization followed by oxidation, as depicted in Scheme 5. The formation of glyoxal from N-fructosylalanine (ARP of glucose and alanine) is in our view best explained via reversed Amadori rearrangement.

The formation of diacetyl can best be explained as originating from an isomerization product of 1-deoxyglycosone, as indicated in Scheme 6. The formation of 2,3-pentanedione can be envisaged as resulting from aldol condensation of diacetyl and formaldehyde, as shown in Scheme 7. Formaldehyde is generated ubiquitously



Scheme 6. Formation of diacetyl.



Scheme 7. Formation of 2,3-pentanedione.

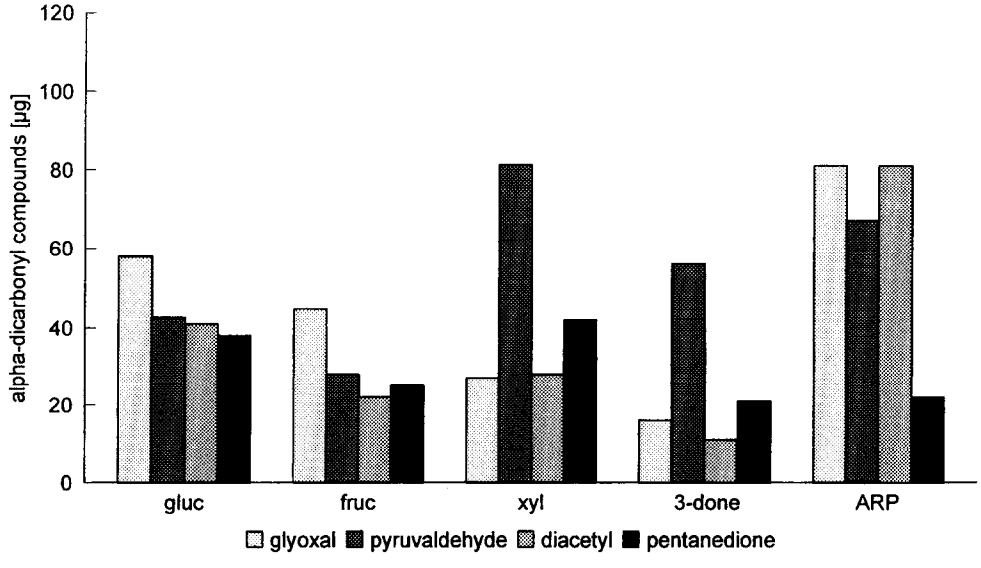


Fig. 1. Formation of alpha-dicarbonyl compounds from carbohydrates and alanine.

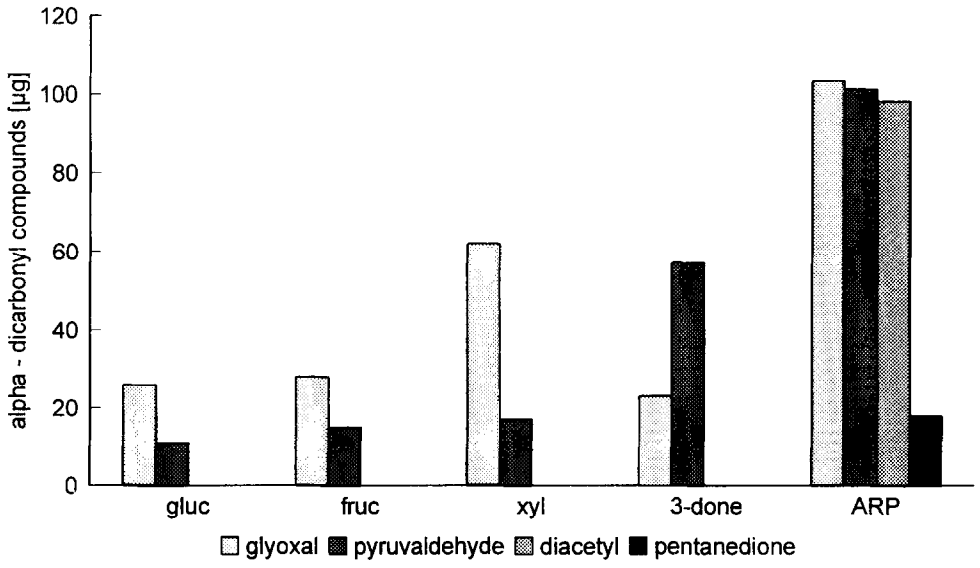
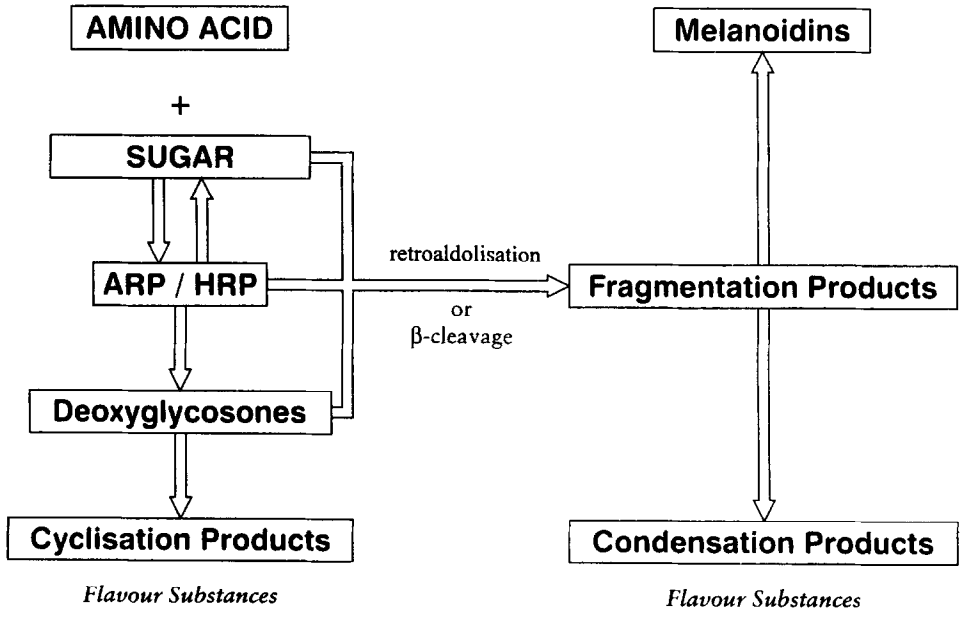


Fig. 2. Formation of alpha-dicarbonyl compounds from carbohydrates in the absence of amine/amino acid.



Scheme 8. The Maillard reaction in flavour formation.

under Maillard reaction conditions, by retro-aldolization of isomerized or fragmented carbohydrates, including deoxyosones (Weenen and Tjan, 1992). This mechanism would imply that the formation of diacetyl and 2,3-pentanedione are coupled, which is in agreement with the observation that diacetyl and 2,3-pentanedione are both formed from various carbohydrates in the presence of amine or amino acid, and are both not formed in the absence of amines or amino acids (see Figs 1 and 2).

The proposed mechanism for the formation of diacetyl is apparently not the only way that diacetyl can be formed, as indicated by the observation that 3-deoxyglucosone, when reacted with alanine or cyclohexyl amine, also generates diacetyl and 2,3-pentanedione, albeit to a lesser extent.

A particularly interesting observation is that both 3-deoxyglucosone and xylose give rise to relatively high yields of pyruvaldehyde. 3-Deoxyglucosone was expected to give, relatively, much pyruvaldehyde, as the proposed mechanism for the formation pyruvaldehyde presumes the formation of 1- and/or 3-deoxyglycosones as intermediates (see Scheme 1). That xylose gives even higher yields of pyruvaldehyde was unexpected. The 1- and 3-deoxyxylosones possibly give retroaldolization more readily, because they are less stable. Xylose contains one carbon less than glucose, hence 1- and 3-deoxyxylosones are expected to be less stable, as several of the bicyclic structures which contribute to the stability of 1- and 3-deoxyglucosone are not possible for 1- and 3-deoxyxylosone (see Scheme 4). Experiments are underway to investigate this hypothesis.

FLAVOUR FORMATION IN THE MAILLARD REACTION

Our experiments with carbohydrate fragmentation suggest that carbohydrate fragments originate from deoxyosones, the intermediate Amadori rearrangement products, as well as from the aldoses directly. Virtually all our observations on carbohydrate cleavage can be explained by retroaldolization and β-cleavage of the starting monosaccharides (or the corresponding imines) or 1- and 3-deoxyglycosones. Direct cleavage of Amadori rearrangement products can, however, not be ruled out. This leads to an overall scheme for flavour formation by Maillard chemistry as depicted in Scheme 8.

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